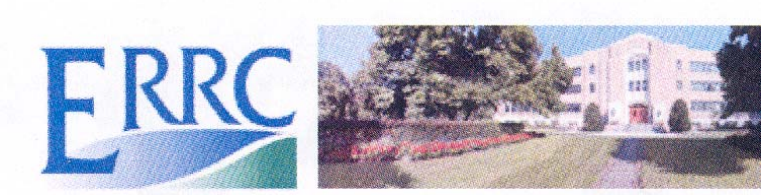

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Effect of pH on the Survival of *Listeria innocua* in Calcium Ascorbate Solutions and on Quality of Fresh-Cut Apples†

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ABSTRACT

Fresh-cut apple slices were dipped in calcium ascorbate (CaA) solution at pH values ranging from 2.5 to 7.0 to inhibit browning. After treatment, the cut apples were stored at 4 and 10°C for up to 21 days. Color and texture of the apples were determined on days 1, 14, and 21. In a separate experiment, the pH of CaA solution was adjusted with acetic acid to six different pH levels, and the solution was inoculated with *Listeria innocua*. The survival of the bacterium and the stability of CaA were determined at 0, 20, and 96 h. The cut apples maintained fresh quality when the pH of the CaA solution was above 4.5, but slight discoloration of apple slices dipped in pH 4.5 solution was observed after 14 days at 10°C. At pH 5.0, the CaA dip maintained the quality of the apples at both temperatures for at least 21 days. The *L. innocua* population was reduced by 4 to 5 log CFU/ml at pH 4.5 after 96 h. At pH 5, the bacterial population in the CaA solution was reduced by approximately 2 log CFU/ml during the same period. The CaA solution was stable at pH 5 for at least 96 h. Reduction of the pH to between 4.5 and 5.0 might reduce the risk of foodborne illness due to consumption of fresh-cut apples treated with a CaA solution contaminated with *Listeria*.

Fresh-cut fruits and vegetables represent a rapidly growing segment of the produce industry. However, there are concerns about microbial food safety and product quality. Some fresh-cut commodities require treatment with inhibitors of enzymatic browning to prevent occurrence of discoloration, which would lower product quality and reduce shelf life *(34)*. Browning inhibitor formulations usually contain ascorbic acid, organic acids, and calcium salts, which are used to maintain firmness of fruits *(30, 34, 35)*. Calcium ascorbate (CaA) is the principal ingredient of a widely used browning inhibitor formulation *(7)*. Browning inhibitor solutions are often expensive or difficult to dispose, and fresh-cut processors may wish to extend the solution life beyond a single day or shift. However, during extended periods of use, these solutions may become contaminated with microorganisms, suspended solids, and soluble solids that originate in the commodities being treated. Depending on time and temperature conditions, the leached solids in these solutions may support the growth of contaminant microorganisms, including human pathogens. This mode of contamination may explain the detection of *Listeria monocytogenes* in sliced apples that resulted in a product recall *(14)*. Conway et al. *(10)* showed that *L. monocytogenes* could grow on fresh-cut apple slices. Currently, there is no processing method that will totally inactivate pathogens on fresh produce without changing the quality of the product *(4)*. *L. monocytogenes* has been associated with a number of outbreaks of foodborne illness *(2, 13, 26)*. Various strains of *Listeria* species can grow over a wide range of temperatures (−1.5 to 45°C) *(18, 23)*.

The problem of preventing bacterial growth in processing aids used in fresh-cut apple processing has not been addressed previously. Earlier studies have considered the reduction and suppression of *Listeria* in broth cultures by reducing the pH or by using preservatives, such as acetic acid, lactic acid, potassium sorbate, sodium benzoate, and sodium propionate *(17, 27, 32)*. Organic acids were reported to be more damaging to the bacteria than HCl; the choice of acid and the concentration of the undissociated compound were considered to be important factors *(1, 5, 6, 37)*.

Research is needed to assess the microbiological risk of extended use of browning inhibitor solutions and to develop the means of suppressing microbial growth, without inducing chemical degradation of browning inhibitors or affecting their antibrowning properties. According to Peri et al. *(29)*, *Listeria innocua* is a suitable choice as a surrogate for *L. monocytogenes*. Hence, the objectives of this research were to study the effects of pH on the survival of *L. innocua* and to investigate pH effects on the stability and functionality of CaA solution in controlling browning and extending the shelf life of fresh-cut apples.

MATERIALS AND METHODS

CaA preparation. CaA was purchased from Shijiazhuang Weisheing Pharmaceutical Co. Ltd. (Shijiazhuang City, China). Solutions containing 7% CaA in distilled water were freshly prepared, and the pH values were adjusted the day before each experiment. The pH was measured using a Select 360 pH meter (Beckman Instruments Inc., Irvine, Calif.) and adjusted with

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17.45 M and 1 M acetic acid (Mallinckrodt, Phillipsburg, N.J.) to values between 2.5 and 7.

Microbiological studies. *L. innocua* 2283 (ERRC culture collection, originally isolated from turkeys at the U.S. Department of Agriculture, Agricultural Research Service, in Ames, Iowa) was used as the surrogate for *L. monocytogenes*. *L. monocytogenes* itself was not used, since it was anticipated that experiments with inoculated CaA solutions would be performed in a pilot plant where human pathogens could not be used. Vials of the frozen (−80°C) culture, which had been prepared from one isolated colony, were defrosted at room temperature, and cells from the vial were grown by inoculating into the culture tubes (25 by 150 mm) containing fresh tryptic soy broth with 0.6% yeast extract (TSBYE; Difco Laboratories, Becton Dickinson, Sparks, Md.) with a loopful of the thawed culture. The culture tubes were incubated overnight at 35°C in a shaking incubator (Innova 4200, New Brunswick Scientific, New Brunswick, N.J.). Before inoculation, the pH values of CaA solutions were adjusted to the desired levels, and the solutions were sterilized using a 0.2-μm filter apparatus (Nalgene, Rochester, N.Y.). Two milliliters of the overnight culture (approximately 10^9 CFU/ml) was used to inoculate 50 ml of CaA solution in 125-ml sterile Erlenmeyer flasks containing sterile magnetic stir bars covered by aluminum foil.

Inoculated CaA solutions in flasks were incubated at 10°C in a refrigerated shaking (182 rpm) incubator (Innova 4230, New Brunswick Scientific). Alternatively, flasks containing these solutions were placed on a multiposition magnetic stirrer (Variomag Magnetic Stirrer, H+P Labortechnik, Oberschleissheim, Germany) set at 300 rpm and installed within a 10°C refrigerator. Samples for enumeration of bacterial populations were taken at 0, 20, and 96 h. Serial dilutions were made with 0.1% peptone water (Difco). Duplicate samples (100 μl) were spread on antimicrobial supplemented polymixin, acriflavine, lithium chloride, ceftazidime, aesculin, and mannitol (PALCAM; Difco) agar and tryptic soy agar plus 0.6% yeast extract (TSAYE; Difco). The plates were incubated at 35°C for 48 h before being hand counted *(23)*. The minimal detection limit was 5 CFU/ml. The bacterial experiments were performed 11 to 12 times.

Measurement of ascorbic acid with HPLC. Samples for high-performance liquid chromatography (HPLC) were taken at 0, 20, and 96 h. Measurement of ascorbic acid has been previously described *(11)*. After proper dilution, CaA solutions were filtered through a 0.45-μm Acrodisc LC 13 PVDF syringe filter (Gelman Sciences, Ann Arbor, Mich.). The filtered samples were placed into 2-ml vials and analyzed using a Hewlett Packard Ti-series 1050 HPLC system (Agilent Technologies, Palo Alto, Calif.). The HPLC system consisted of an autosampler, an integral photodiode-array detector, an auto injector, and a Hewlett-Packard Rev. A02. 05 Chemstation. The injection volume was 20 μl. Separation of compounds was achieved with an Aminex HPX-87H organic acid column (300 by 7.8 mm) fitted with a microguard cation H^+ cartridge (Bio-Rad Laboratories, Hercules, Calif.) and eluted with a mobile phase of 5 mM sulfuric acid at a flow rate of 0.5 ml/min. Column temperature was maintained at 30°C using a column heater (Bio-Rad Laboratories). The ascorbic acid peak was monitored at 245 nm and quantified by analysis of an ascorbic acid standard.

Preparation of the apple samples. Granny Smith apples (*Malus pumila*) were purchased from a local grocery store and used within 7 days. They were kept at the refrigerator temperature in covered containers until they were used. They were dipped in a 200-ppm chlorine solution (sodium hypochlorite) for 90 s. Apples were drained and air dried on sterile aluminum foil. Each apple was cut into 10 pieces by using a Westmark wedger and corer (Westmark Divisorex, Herscheid, Germany), which was cleaned and sanitized by dipping in approximately 1% sodium hypochlorite solution for approximately 20 min. For color testing, cross sectional slices of apples were used. Residual core tissue remained on some slices. Apple slices or cross-cuts were dipped for 90 s in the CaA solutions at different pH values ranging from 2.5 to 7.0. After dipping, the cut apple slices were drained, packaged in a proprietary film bag designed for fresh-cut fruit, heat sealed (model no. MP-16, Midwest Pacific impulse sealer, Foster City, Calif.), and stored at 4 and 10°C. Color and texture analyses were performed at 0, 14, and 21 days of the storage period. There were a total of five to six measurements for each treatment (pH).

Color measurement. The slices were able to fit over the colorimeter aperture. A Hunter Miniscan XE colorimeter with a 26-mm measuring aperture (Hunter Associates Laboratory, Reston, Va.) was used to assess lightness (L* values), redness-greenness (a* values), and blueness-yellowness (b* values) of the apple slices *(12)*. D65/10° was the illuminant or viewing geometry. Samples were measured following calibration with the standard white and black plates.

Texture measurement. Texture was determined using the TA.XT2i Texture Analyzer (Texture Technology Corp, Scarsdale, N.Y.) and a TA-212 cylinder probe (11 mm in diameter) *(12)*. The probe moved down at 2 mm/s to 1 cm below the surface of samples. Maximum force was recorded using the Texture Expert software (version 1.22, Texture Technology Corp, Scarsdale, N.Y.). There were five to six samples per treatment.

Statistical analysis. Linear regression was used for evaluation of the bacterial survival data. The data from texture and color measurements of apple slices were examined by analysis of variance (ANOVA) to determine the effects and interactions of storage time, temperature, and pH on the responses (maximum force, L*, a*, and b*). Mean separations were performed using the Bonferroni least significant difference technique.

RESULTS

Survival of *L. innocua* in CaA solution. The bacterial survival results show that when the pH was lowered, the population of *L. innocua* in the CaA solution decreased. Reductions of *L. innocua* populations were greater at pH 2.5 and 3.5 than at higher pH values; at these lower pH values, the *L. innocua* population was reduced to below the minimum detectable limit (<5 CFU/ml) within 20 h at 10°C (Table 1). At pH 4.5, the *L. innocua* population was reduced by approximately 5 to 6 log within 96 h, whereas at pH 5.0, the *L. innocua* population in CaA solution decreased by 0.5 to 0.75 log in 20 h and by 1 to 2 log in 96 h. At pH 6.0 and 7.0, the *L. innocua* population did not change significantly in 96 h at 10°C (Table 1). Using a shaker or a stir plate during incubation gave statistically similar results ($P > 0.05$) at pH 5.0, 6.0, and 7.0.

The statistical analysis revealed no difference between agar types (PALCAM versus TSAYE) for recovering *L. innocua* ($P > 0.09$). Incubation time and pH had statistically significant effects on survival. The interaction between time and pH also was statistically significant. The effects of time and pH on log (CFU) can be expressed as follows: $\log(\text{CFU}) = 4.99 - 2.02 \times \text{time} + 0.37 \times \text{pH} + 0.30 \times \text{time} \times \text{pH}$ ($R^2 = 0.395$). The model is statistically signif-

TABLE 1. *Effect of pH on survival of* Listeria innocua *in calcium ascorbate solutions at 10°C for different storage times*

pH	*Listeria innocua* population (log CFU/ml) by storage time[a]		
	0 h	20 h	96 h
2.5	3.5 ± 4.0 (4)	<0.7 (4)	<0.7 (4)
3.5	5.6 ± 1.7 (4)	<0.7 (4)	<0.7 (4)
4.5	7.3 ± 1.0 (26)	6.5 ± 1.0 (26)	1.5 ± 1.3 (26)
5.0	7.6 ± 0.6 (30)	7.1 ± 0.9 (30)	5.9 ± 1.6 (30)
5.5	7.3 ± 1.1 (10)	7.0 ± 1.5 (10)	7.9 ± 0.1 (10)
6.0	7.5 ± 0.6 (24)	7.3 ± 0.5 (24)	7.0 ± 2.4 (24)
7.1	7.5 ± 1.0 (38)	6.8 ± 1.3 (38)	7.7 ± 1.3 (38)

[a] Survival of *L. innocua* measured as average of log CFU/ml ± SD with number of measurements shown in parentheses. Detection limit was 5 CFU/ml.

icant ($P < 0.001$). This indicates a linear decrease of *L. innocua* population (log values) with increasing time (hours) and a linear increase with increasing pH. *P* values for the *F* statistic from ANOVA tables were $P < 0.0001$ for pH, time, and pH × time.

Apple quality changes. Instrumental measurements of color provide an objective assessment of browning at the cut surfaces of apple slices. On day 1, apple slices treated with CaA solutions at pH values of 2.5 and 3.5 had lower L* and higher a* values than those treated at higher pH values (Table 2). The lower L* and higher a* values suggest that these apple slices may have had more initial browning than those treated at other pH values. The b* values were highest with apple slices treated with a pH 4.0 CaA solution. The firmness of apple slices was lowest with the pH 2.5 treatments.

After 14 days of storage at 4°C, apple slices treated with pH 3.5 and 4.0 CaA solutions had lower L* and higher a* values than those treated at other pH values. This is an indication of browning (Table 3). There were no significant differences in L* and a* values between apples treated at pH values of 4.5 to 7.0. Slices treated with a pH 4.0 CaA solution had the highest b* values. The firmness of apple slices dipped in CaA solutions at pH values of 2.5 to 4.0 tended to be lower than that of slices treated at higher pH values. After 14 days at 10°C, apple slices treated at pH values of 3.5 and 4.0 had lower L* values and higher a* and b* values than those treated at other pH values. Slices treated at a pH of 2.5 had higher a* values than those treated at pH values above 4.0. Apple slices treated at pH values below 4.5 had significantly lower firmness than those treated at or above pH 4.5 ($P < 0.01$), although differences were often not statistically significant among samples at pH values ranging from 2.5 to 4.5.

After 21 days of storage at 4°C, apple slices treated at pH 3.5 and 4.0 had lower ($P < 0.01$) L* and higher ($P < 0.01$) a* and b* values than those treated at other pH values (Table 4). The a* values of slices treated at pH 2.5 were lower than those treated at pH 3.5 and 4.0 but higher than those treated at pH above 4.0. These instrumental color data and the photographs indicate that apple slices treated at pH

TABLE 2. *Effect of pH on color and texture of apple slices dipped for 90 s in 7% calcium ascorbate solutions after 1 day of storage at 4°C[a]*

pH	L* (lightness)[b]	a* (green/red)[c]	b* (blue/yellow)[d]	Texture (kg)
2.5	75.7	0.1	20.8	4.5
3.5	71.7	0.9	22.8	6.3
4.0	77.2	−2.3	26.2	6.3
4.5	77.9	−3.4	22.8	6.2
5.0	78.5	−3.4	25.8	5.8
5.5	77.8	−3.6	22.4	5.8
6.0	79.5	−3.7	23.8	6.4
6.5	80.8	−3.8	21.9	6.8
7.0	80.0	−3.4	21.8	5.2
LSD[e]	6.3	2.5	4.3	0.7

[a] Five to six measurements for each treatment.
[b] Zero equals black; 100 equals white.
[c] Negative a* values indicate greenness; positive a* values indicate redness.
[d] Negative b* values indicate blueness; positive b* values indicate yellowness.
[e] Least significant difference at $P < 0.01$.

TABLE 3. *Effect of pH on color and texture of apple slices dipped for 90 s in 7% calcium ascorbate solutions after 14 days of storage at 4 and 10°C[a]*

Temp. (°C)	pH	L* (lightness)[b]	a* (green/red)[c]	b* (blue/yellow)[d]	Texture (kg)
4	2.5	79.5	−1.3	19.1	3.5
	3.5	68.9	3.9	24.0	5.3
	4.0	66.7	6.9	27.0	4.6
	4.5	80.3	−3.2	25.7	6.1
	5.0	80.0	−3.3	23.3	7.1
	5.5	80.9	−3.1	22.6	7.7
	6.0	80.3	−3.1	21.5	6.6
	6.5	80.2	−3.4	24.6	5.8
	7.0	81.2	−3.1	24.1	6.2
	LSD[e]	6.4	3.0	4.3	1.6
10	2.5	76.8	−0.1	22.2	3.6
	3.5	61.5	9.9	33.5	3.8
	4.0	59.6	9.7	34.0	3.8
	4.5	78.0	−3.4	25.4	7.3
	5.0	70.8	−3.3	24.7	7.2
	5.5	79.4	−3.4	26.5	6.5
	6.0	81.1	−3.5	27.3	7.0
	6.5	77.9	−3.9	27.8	5.4
	7.0	78.6	−3.8	27.6	7.3
	LSD[e]	6.1	2.6	3.5	1.2

[a] Five to six measurements for each treatment.
[b] Zero equals black; 100 equals white.
[c] Negative a* values indicate greenness; positive a* values indicate redness.
[d] Negative b* values indicate blueness; positive b* values indicate yellowness.
[e] Least significant difference at $P < 0.01$.

and 4.0 had the most browning, followed by those treat- at pH 2.5 (Tables 3 and 4 and Fig. 1). Apple slices ated at pH 2.5 and 3.0 had lower firmness than those ated at other pH values. Similar results were also ob- ved in the color attributes (L*, a*, b*) after 21 days at °C. However, slices treated at pH 4.5 had higher a* val- s than those treated at pH values above 4.5. The higher values observed at pH 4.5 indicate that these apple slices d more browning than those treated at a pH above 4.5. ple slices treated at pH 4.0 or below tended to have ver firmness than those treated at pH values above 4.0.

Overall, pH and storage time had statistically signifi- nt ($P < 0.001$) effects on all three-color parameters and mness (Tables 3 and 4). ANOVA showed that in each se there was evidence of significant interactions among me, temperature, and pH (Table 5). The instrumental color easurements were in agreement with photographic obser- tions of appearance (Fig. 1).

Stability of CaA solution. Ascorbic acid levels in the aA solutions were not consistently affected by the solution H between 4.5 and 7.0 (Table 6). Ascorbic acid levels anged little during the 96-h storage period at 10°C. Also, tration (0.2-μm filter) did not alter the levels of ascorbic id in the solutions.

DISCUSSION

Our results clearly show the suppression of *L. innocua* rvival in the CaA solutions by acidification to low pH ith acetic acid. Acidification of coleslaw and cabbage ice resulted in reductions in *L. monocytogenes* popula- ons *(8, 15)*. In culture media, acetic acid has a more potent tilisterial effect than lactic acid, which is more inhibitory an hydrochloric acid *(1, 19, 22)*. The inhibition of *L. mon- ytogenes* by acids is caused not by a decrease in the in- acellular pH by itself but rather by the specific effects of rticular undissociated acids on metabolic or other physi- ogical activities *(19)*. We found that the *L. innocua* pop- ation decreased rapidly in CaA solution at pH 2.5 and 5, with no detectable survivors within 20 h. These results gree with Conte et al. *(9)*. On the other hand, George et . *(16)* detected *L. monocytogenes* growth at pH 4.62, 5.45, nd 5.23 at 10, 7, and 4°C, respectively, between 11 and 1 days. This result may be due to their use of a high- utrient environment, high inoculum levels, and hydro- nloric acid as the acidulant. McKellar et al. *(25)* reported iat reducing the pH to 5.5 had a minimal effect on growth f *L. monocytogenes*, but the population decreased notice- bly when the pH was below 5.5. Our finding that pH 6.0 nd 7.0 had no effect on the *L. innocua* population is con- stent with the results of Marc et al. *(23)*, George and Lev- tt *(15)*, and Bereksi et al. *(3)* with *L. monocytogenes*. It night also be due to the closeness of these pH values to ne optimum pH for *Listeria* growth.

Enzymatic browning at cut surfaces of apple slices can e monitored by measuring changes in reflectance L* and * values *(33)*. The reflectance parameters were measured n our study to evaluate the effectiveness of CaA dips in suppressing polyphenol oxidase activity and subsequent browning of the sliced apples.

TABLE 4. *Effect of pH on color and texture of apples slices dipped for 90 s in 7% calcium ascorbate solutions after 21 days of storage at 4 or 10°C*[a]

Temp. (°C)	pH	L* (lightness)[b]	a* (green/red)[c]	b* (blue/yellow)[d]	Texture (kg)
4	2.5	76.2	−0.9	21.9	4.0
	3.5	62.7	9.0	33.5	5.7
	4.0	63.0	7.9	34.0	4.3
	4.5	80.5	−3.1	25.4	7.1
	5.0	79.9	−3.0	24.7	6.2
	5.5	80.5	−3.4	27.0	7.1
	6.0	79.7	−3.9	27.3	7.5
	6.5	78.4	−4.0	27.8	6.5
	7.0	80.0	−3.7	27.6	5.8
	LSD[e]	5.6	2.7	3.9	1.2
10	2.5	75.6	1.0	25.1	3.8
	3.5	63.1	9.0	32.4	4.7
	4.0	69.4	4.0	29.7	5.5
	4.5	75.9	−1.6	23.9	7.1
	5.0	78.5	−3.4	27.1	6.7
	5.5	78.3	−3.4	28.5	7.4
	6.0	80.3	−3.1	28.3	6.5
	6.5	78.4	−3.6	28.6	7.2
	7.0	77.6	−3.0	27.2	6.8
	LSD[e]	5.9	2.9	4.9	1.7

[a] Five to six measurements for each treatment.
[b] Zero equals black; 100 equals white.
[c] Negative a* values indicate greenness; positive a* values indicate redness.
[d] Negative b* values indicate blueness; positive b* values indicate yellowness.
[e] Least significant difference at $P < 0.01$.

Any use of pH reduction to suppress survival of *Listeria* in CaA solution must take into account the detrimental effect of reducing the pH of this solution on apple quality. Our results show enhanced apple browning (especially in core tissue) at decreasing pH between 4.5 and 3.5 (less browning at pH 2.5 because of acid inhibition of polyphenol oxidase) and textural deficiencies at a pH of less than 4.5 (Tables 2 through 4). Cut apples can be treated at pH 4.5 and stored at 4°C for 21 days and at 10°C for 14 days with no negative effects on the quality (Fig. 1). However, at 10°C the apples treated at pH 4.5 might show more browning than the apples treated at pH 5.0 during longer storage. Also, temperatures in supermarket display cases or in home refrigerators can be excessively high, thus permitting accelerated browning of a marginally stable product. Thus, pH 4.5 is a critical point for the preservation of apple quality. Even if pH 4.5 is much more lethal to *Listeria* than pH 5, it would be unwise to recommend the lower pH. It would be better to adjust the pH of CaA solution to 5.0 or some intermediate pH value rather than to 4.5 for the treatment of cut apples. The combination of ascorbic acid and

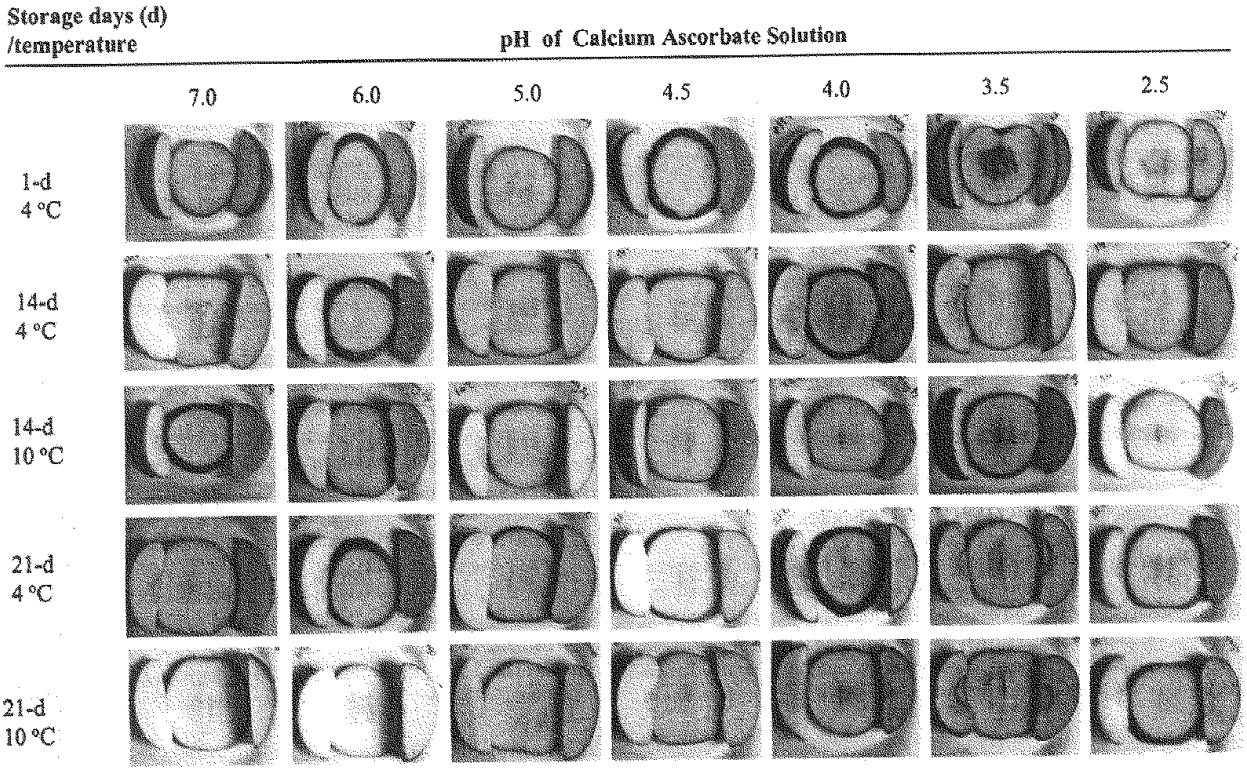

FIGURE 1. *Effect of pH and storage time on browning of apple slices treated with 7% calcium ascorbate.*

calcium has been shown previously to be effective in inhibiting browning *(31, 36)*. In our study, the high concentration of CaA solutions (7%) prevented browning at pH 5.0 (Tables 2 through 4 and Fig. 1).

Hydrogen ion can be considered as a noncompetitive inhibitor of apple polyphenol oxidase for pH lower than 5 *(20, 38)*. According to Janovitz-Klapp et al. *(20)*, inhibition of polyphenol oxidase increased as the pH was lowered from 5 to 3.6, and the results indicated that the undissociated acid was mainly responsible for inhibition. At pH values below 3, the enzyme is effectively inhibited *(24)*. However, the activity at pH 3 still represented 40% of the maximum activity *(21)*. Thus, control of enzymatic browning by acidification is difficult except at very low pH *(28)*. Therefore, our results indicating partial suppression of browning at pH 2.5 are consistent with these findings. Even if browning and *Listeria* survival can be suppressed by low pH, such treatment is not feasible, since the firmness of the sliced apples is rapidly lost (Tables 2 through 4).

Lowering the pH of CaA solution to 5.0 by acetic acid addition resulted in a reduction in the *L. innocua* population of approximately 2 log during 96 h at 10°C. The color of the apple slices treated with the pH 5.0 solution to control browning was stable for up to 21 days at 4 and 10°C. A greater population reduction could be obtained at pH 4.5 but with the risk of some browning under conditions of temperature abuse. Thus, adjustment of the CaA solution to pH 5.0 or some intermediate pH between 4.5 and 5.0 might be an acceptable means of controlling *Listeria* without adversely affecting appearance or texture of the sliced apples. Further evaluation of this approach, with *L. monocytogenes* used as the target organism and commercial scale tests to confirm the quality of the sliced apples by sensory evaluation, would be useful.

TABLE 5. P *values for the* F *statistic from ANOVA tables*[a]

	Color			
	L*	a*	b*	Texture
pH	<0.001	<0.0001	<0.0001	<0.0001
Time	0.0210	<0.0001	<0.0001	<0.0001
Temperature	0.0051	0.1225	<0.0001	0.0163
Time × pH	<0.0001	<0.0001	<0.0001	<0.0001
Temperature × pH	0.6408	0.0106	0.0133	0.0091
Time × temperature × pH	0.0094	<0.0001	0.0058	0.8283

TABLE 6. *Concentration of ascorbic acid in 7% calcium ascorbate solutions as affected by pH and storage at 10°C*[a]

	Ascorbic acid (mg/g) by storage time		
pH	0 h	20 h	96 h
4.5	65.8	58.5	57.2
5.0	54.4	56.1	56.3
5.5	61.6	53.4	56.6
6.0	57.7	54.0	55.9
7.0	57.9	56.6	54.2
LSD[b]	5.9	5.4	3.6

[a] Ascorbic acid measured using high-performance liquid chromatography; four measurements for each treatment.
[b] Least significant difference at $P < 0.01$.

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